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Common hemostasis and inflammation gene variants and venous thrombosis in older adults from the Cardiovascular Health Study

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SUMMARY

Background/Objectives—Age-related changes in blood coagulation and fibrinolysis are associated with increased risk of thrombotic events. Inherited deficiencies of coagulation proteins, such as factor V Leiden and prothrombin G20210A, explain a small fraction of venous thromboembolic disease (VTE). Additional genetic factors likely underlie the etiology of VTE, some of which may become manifest at older ages.

Methods—We tested 290 common SNPs within 51 thrombosis and inflammation genes for association with VTE in the Cardiovascular Health Study, a large, prospective cohort of older adults followed for up to 12 years.

Results—There were 184 VTE events that occurred at mean age of 78 years. TagSNPs within four genes encoding factor XIII subunit A (*F13A*), factor VII activating protease (*HABP2*), protease activated receptor -1 (*F2R*), and the urokinase receptor (*PLAUR*) showed the strongest evidence for association with VTE, with each gene having a global p-value <0.05 and at least one tagSNP false discovery rate (FDR) *q*-value <0.05. The *rs*3024409 variant allele of *F13A1* was associated with 1.66-fold increased risk of VTE, while the minor alleles of *HABP2 rs*6585234 and *rs*3862019, *F2R rs*253061 and *rs*153311, and PLAUR *rs*344782 were each associated with lower risk of VTE (hazard ratios in the range of 0.49 to 0.66). Consistent with the observed protective association on VTE risk, the *HABP2 rs*3862019 variant allele was also associated with lower activity levels of coagulation factors VIII, IX, X, and plasminogen. We also confirm previously reported associations between common variants of the coagulation factor II, V, VIII, XI, alpha-fibrinogen, and protein C genes and risk of VTE.

Conclusions—These findings suggest that several novel common coagulation gene variants may be related to risk of VTE in older adults. Further studies in older adults are needed to validate these findings and assess functional molecular mechanisms.

Keywords

venous thrombosis; factor XIII; factor VII activating protease; genetics

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INTRODUCTION

Venous thromboembolism (VTE) occurs disproportionately among older adults [1]. The reasons likely involve increases in both intrinsic blood coagulation potential that occur with age and presence of acquired co-morbid conditions that predispose to thrombosis, such as obesity, surgery, cancer, and immobility.

Family studies suggest the existence of genes that jointly influence thrombosis susceptibility and plasma coagulation and fibrinolytic factor levels [2]. The strong influence of genetic factors on plasma coagulation factor levels persists into older adulthood [3]. Inherited deficiencies of antithrombin, protein C, and protein S, and prothrombotic mutations such as Factor V Leiden and prothrombin G20210A, predispose to VTE [4]; but such familial thrombophilic disorders explain only a small fraction of VTE occurring in the community [5-7].

Recent observational studies of middle aged to older adults suggest that coagulation and inflammation gene sequence variants that are even more common in the population (allele frequencies in the range of 10%-40%) may contribute to VTE risk [8-10]. Whether such common thrombosis-related gene variants influence VTE occurrence has not been previously examined in prospective studies of community-dwelling older adults. Therefore, we screened a large panel of common single nucleotide polymorphism (SNPs) within candidate coagulation and inflammation genes for association with VTE in the Cardiovascular Health Study (CHS), a large, well-characterized prospective population-based study of older adults.

METHODS

Study participants and data collection

The CHS is a prospective population-based cohort study of U.S. adult men and women 65 years and older recruited from four field centers: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania [11]. Those eligible to participate included all persons 65 years of age or older living in the household of each individual sampled. The original cohort (n=5,201) was recruited from 1989 to 1990. A second African-American (AA) cohort (n= 687) was recruited between 1992 and 1993. Of the total CHS cohort (n=5,888), 4,925 participants identified themselves as European-American (EA), 924 as AA, and 39 as “other.” Local institutional review committees approved the CHS protocol, and all subjects gave informed consent.

VTE case ascertainment and inclusion criteria

VTE was defined as the occurrence of validated deep vein thrombosis or pulmonary embolism from baseline through December 31, 2001, as previously reported [12] and described under Supplemental Methods. Of the total CHS cohort (n=5,888), 206 participants developed incident VTE during follow-up. After excluding 502 subjects who did not consent to DNA testing and/or did not have aliquots of DNA available for genotyping, there were 5,386 CHS participants eligible for the current study. These included 184 cases and 5,202 non-cases of VTE.

Plasma coagulation factor measurement

Fibrinogen, factor VII, and factor VIII coagulant activity were measured using citrated plasma in 5,024 EA participants from the original CHS cohort. Factor IX activity, factor X activity, and plasminogen levels were measured on a cross-sectional sub-sample of 340 eligible participants from the original cohort. Plasma fibrinogen was determined using the von Clauss method. Factor VII, VIII, IX, and X activity were measured using 1-stage clotting assays and reported as percent of a normal plasma pool [13,14]. Plasminogen was measured by rate chromogenic assay [15].

Candidate gene SNP selection and genotyping

We selected 51 coagulation-, fibrinolysis-, and inflammation-related candidate genes for analysis, as described under Supplemental Methods and summarized in Supplemental Table 1. We selected a set of tagSNPs that efficiently capture common patterns of genetic variation in the genomic regions spanning 5 kb upstream of the transcription start site and 3 kb downstream of the transcription end site of our genes of interest using the LDselect algorithm [16]. SNPs were selected such that all known common variants in each gene (defined as minor allele frequency or MAF ≥10%) are correlated with a tagSNP with r^2 of >0.8 in the European SNP discovery panels from SeattleSNPs (http://pga.mbt.washington.edu/) or International HapMap project (hhttp://www.hapmap.org). Neither Factor V Leiden nor prothrombin G20210A were typed as part of the current study because their allele frequencies are <10%. For some LD bins, multiple tagSNPs were typed in the case of assay failure. These redundant tags (r^2 >0.8) were removed prior to analysis. A total of 375 SNPs with MAF 10% were successfully genotyped across the 51 candidate genes. After removing 85 SNPs that had a pair-wise r^2 >0.8 with at least one other SNP, 290 non-redundant tagSNPs remained in the final analytic data set. Genotyping was performed as described under Supplemental Methods.

Statistical analysis

Our underlying hypothesis was that one or more common SNPs in genes related to coagulation, fibrinolysis, and inflammation are associated with risk of VTE in older adults. In our primary analysis, we included all incident and recurrent cases of VTE, and restricted our analysis to self-identified whites to minimize confounding due to population structure. We assessed the association between each tagSNPs and time to VTE during follow-up using Cox proportional hazards regression, minimally adjusting for age and sex. We assessed tagSNP associations with continuous measures of plasma coagulation factors using linear regression, using appropriately transformed dependent variable, adjusted for age and sex. For time-to-event models, covariate-adjusted hazard ratios were estimated from the regression coefficients, assuming a constant effect size for each additional copy of the minor allele. For autosomal markers, SNP genotype was coded 0/1/2 the number of copies of the minor allele assuming an additive inheritance model. For X-chromosomal markers, male genotypes were coded 0/1 and female genotypes were coded 0/0.5/1 (because of X-chromosome inactivation in women) and the regression model adjusted for sex. Individual SNP associations with VTE were considered nominally significant at a *p*-value threshold <0.05. To formally assess the statistical significance of our results, we accounted for multiple hypothesis testing in our primary analysis in two ways: (1) at the level of each of the 51 candidate genes, we computed a global gene-based *p*-value using a permutation-based, and (2) at the level of the 290 individual tagSNPs using the false discovery rate (FDR) or *q*-value method (see Supplemental Methods).

We performed several sensitivity analyses: (a) In to addition to age and sex, Cox regression models were adjusted for smoking, BMI, and cancer (to assess the possibility that any observed SNP association might be mediated through other VTE risk factors). (b) We repeated our primary analysis after excluding 384 participants who had a self-reported a history of VTE and/or were taking warfarin anticoagulants at the time of CHS study entry. (c) We included self-reported African-American participants, additionally adjusting for race. (d) For X-linked genes, we confirmed any genotype-phenotype associations by additionally repeating the regression models separately in men and women.

RESULTS

Participant characteristics

The mean age of the 5,386 eligible CHS study participants was 73 years. Fifty-eight percent were women, and 16% were African-American. Participant baseline characteristics stratified

by race are shown in Table 1. During a mean (median) follow-up of 9.4 (11.6) years, 184 (3%) developed VTE. The mean age at onset of VTE was 78 years. Of the 184 VTE cases, 105 (57%) were female, 40 (22%) were African-American, and 31 (17%) reported a previous history of VTE. When classified according to presence or absence of precipitating risk factors, 77 VTE events were idiopathic and 107 occurred secondary to other causes (cancer, recent trauma, surgery, or immobility).

Candidate gene SNP associations with VTE

Of 51 candidate genes screened, 12 genes had at least one tagSNP nominally associated with VTE risk (p <0.05), including 8 genes with multiple tagSNPs nominally associated with VTE risk (Table 2). Four candidate genes (*HABP2*, *F2R*, *PLAUR*, and *F13A1*) had global gene-based *p*-values for association with VTE that were less than 0.05, and the *F8* gene had a global p-value of 0.06. Of 290 tagSNPs tested, a total of 30 SNPs (~10%) were nominally associated with risk of VTE.

When the individual tagSNPs were corrected for multiple comparisons using the FDR method, 6 tagSNPs were significantly associated with VTE risk at a *q*-value threshold of <0.20 (Table 3). The tagSNP mostly strongly associated with VTE risk was *F13A1 rs*3024409, located in intron 7 of the factor XIII A subunit gene (hazard ratio = 1.66; 95%CI 1.31 - 2.11; *p*-value $=10^{-5}$; FDR *q*-value = 0.002). The minor alleles of 5 other intronic tagSNPs, including *rs*3862019 and *rs*6585234 of the factor VII activating protease gene (FSAP) gene (*HABP2*), *rs*253061 and *rs*153311 of the protease activated receptor (PAR)-1 gene (*F2R*), and *rs*344782 of the urokinase receptor gene (*PLAUR*), were associated with lower risk of VTE (hazard ratios in the range of 0.49 to 0.66). None of the inflammation gene variants within our panel were significantly associated with VTE risk.

Repeating the individual SNP association analyses by (a) restricting to those without a prior history of VTE or baseline warfarin use, or (b) additional adjustment for BMI, smoking, and cancer, or (c) including African-American participants did not substantively affect any of these results. In additional analyses, there was no evidence that the SNP-associated risks of VTE differed among those with first versus recurrent VTE, nor among those VTE cases classified as idiopathic versus secondary to cancer, recent trauma, surgery, or immobility (data not shown). When stratified by race, there was no evidence that the risk of VTE associated with *F13A1 rs*3862019, *HABP2 rs*3862019, *F2R rs*253061, *F2R rs*153311, or *PLAUR rs*344782 differed between whites and African-Americans. In contrast, the *HABP2 rs6585234* showed little evidence of VTE association in African-Americans (HR = 1.08; 95% CI 0.68 – 1.71). When analyzed separately by gender, the *rs*1800921 factor VIII variant encoding the Glu1241Asp polymorphism was associated with a HR of 0.37 (95%CI 0.14 – 1.03) in men. Women heterozygous for the Asp1241 variant allele had an HR of 0.80 (95%CI 0.48 – 1.35), while women homozygous for the variant allele had an HR of 0.32 (95%CI 0.05 – 2.35).

Assessment of VTE-associated SNPs in *HABP2* and *F8* with coagulation factor levels

Because of its ability to activate or cleave various substrates (coagulation factor VII, pro-urokinase, fibrinogen, and platelet derived growth factor-BB) in vitro [17], we further explored potential mechanism underlying the observed *HABP2*– VTE associations by assessing genotype associations with plasma coagulation factor levels previously measured at baseline in CHS (Table 4). Factor VII, VIII, and fibrinogen levels were measured in the entire cohort, while factor X, IX, and plasminogen levels were measured in a selected sample of 345 CHS participants. The *rs*3862019 *HABP2* SNP associated with decreased risk of VTE was associated with lower coagulation factor VIII, IX and X activity levels, and lower plasminogen levels, particularly among homozygotes for the rare allele. In contrast, there was no association between *rs*3862019 genotype and levels of factor VII activity or fibrinogen. Since factor IX,

X, and plasminogen were measured at baseline in only a fraction of the CHS cohort, we were unable to assess whether the addition of these intermediate traits as covariates in the Cox regression model results in any attenuation of the association between *HABP2* genotype and risk of VTE.

Because of the previous reported association between *F8 rs*1800921 and lower factor VIII levels [18], we assessed the association between *F8* genotype and factor VIII activity in CHS. The *F8 rs*1800291 coding variant associated with decreased VTE occurrence was associated with lower circulating factor VIII in both men and women (Table 4). Nonetheless, in a Cox regression model containing both factor VIII level and *F8* genotype, there was little change in VTE risk estimate for *rs*1800291 when adjusted for factor VIII activity levels (HR=0.69; 95% 0.49 – 0.96; p=0.03 with factor VIII:C adjustment versus HR=0.67; 95% 0.48 – 0.94; p=0.02 without factor VIII:C adjustment).

DISCUSSION

We identified several common genetic variants associated with VTE risk in older adults. TagSNPs within genes encoding factor XIII subunit A (*F13A*), factor VII activating protease (*HABP2*), protease activated receptor-1 (*F2R*), and the urokinase receptor (*PLAUR*) showed the strongest evidence for association with VTE.

Newly identified coagulation gene SNPs putatively associated with VTE risk in CHS

Upon activation by thrombin, factor XIII A catalyzes the formation of cross-links between fibrin molecules, thus stabilizing the fibrin clot. The *rs*3024409 variant of the factor XIII A subunit gene results in a G/T substitution in intron 7 and is in strong linkage disequilibrium with at least one other known *F13A1* SNP (*rs*3024425 in intron 8; r^2=1.0). *FXIIIA1 rs*3024409 is not in linkage disequilibrium with the factor XIIIA Val34Leu polymorphism (*rs*5985) that has been reported to be associated with a modest reduction in VTE risk [19]. In a recent nested case-control analysis that included participants from both CHS and the Atherosclerosis Risk in Communities (ARIC) study, Val34Leu was not significantly associated with VTE [20]. Nine other common *F13A1* variants were not associated with VTE risk in post-menopausal women [8], but the *rs*3024409 and *rs*3024425 polymorphisms were not specifically tagged by the 9 tagSNPs assessed (maximum pair-wise r^2 =0.3).

A Gly534Glu polymorphism (Marburg I) of FSAP has been associated with lower proteolytic activity towards pro-uPA and risk of both arterial and venous thrombotic disease [17]. The Marburg I polymorphism has a population allele frequency of <5%, and therefore was not assessed in the current study. The *HABP2 rs*6585234 and *rs*1885436 variants are located within introns and are in strong to moderate LD (r^2>0.64) with at least 10 other common intronic SNPs. Despite the lack of any obvious effect on *HABP2* gene or FSAP protein function, the observed association of these *HABP2* intronic SNPs with levels of several intrinsic coagulation pathway components (factors X, IX, and VIII) lend further support for a potential physiologic role of FSAP in regulation of hemostasis and thrombosis [17].

Urokinase plasminogen activator receptor (uPAR) is a GPI-linked protein expressed on leukocytes that mediates fibrinolysis through a urokinase-dependent mechanism. Soluble uPAR levels are increased in patients with paroxysmal nocturnal hemoglobinuria (PNH), who are prone to developing venous thrombosis [21]. The *PLAUR rs*344782 variant associated with VTE is located in intron 1 is in perfect linkage disequilibrium (r^2=1.0) with 4 other intronic *PLAUR* polymorphisms: *rs*344787, *rs*344785, *rs*344784, and *rs*344783. *PLAUR rs*344782 and *rs*344785 are located ~500 bp upstream and ~ 100 bp downstream, respectively, of a transcription start site for an alternatively spliced *PLAUR* transcript.

Protease-activated receptor 1 (PAR-1) is the main thrombin receptor on platelets and vascular endothelial cells and is encoded by the *F2R* gene. The minor allele of *rs*153311 has been associated with lower PAR-1 platelet receptor density and lower response to the SFLLRN peptide agonist [22]. In the Paris Thrombosis Study, another *F2R* variant (the insertion allele of the −506 I/D polymorphism) was associated with lower risk of VTE in men (odds ratio = 0.52; 95% CI 0.32 to 0.82, $P<0.01$), but not in women [23]. In CHS, there was little evidence of VTE association for a SNP (*rs*2227750) that tags the −506 I/D polymorphism (hazard ratio =1.15, 95%CI 0.91 – 1.44; p=0.23). However, we did find a significant gender interaction between the *F2R* intron 1 *rs*253061 polymorphism and VTE risk in CHS [HR 0.77 (95% 0.55 – 1.09) in women and 0.38 (95% 0.23 – 0.62) in men; *p* for interaction = 0.02].

Results in CHS for coagulation gene SNPs previously associated with VTE

In Supplemental Table 2, we summarize the VTE association results in CHS for other coagulation gene variants that have been reported as putative VTE susceptibility loci in other studies. The Asp1241Glu variant of factor VIII (*rs*1800921) has been associated with lower factor VIII levels [18] and also with lower risk of VTE in men but not in women [24]. In CHS, we observed associations with lower factor VIII activity and decreased VTE risk in both sexes. In a genome-wide screen of two combined case-control studies, SNPs in the genes encoding antithrombin (*SERPINC1*), platelet collagen receptor glycoprotein VI (*GP6*), and factor XI (*F11*) were robustly associated with risk of venous thrombosis [9]. Our results and those of Smith et al [8] confirm the association between the *F11* variant and increased risk of VTE. The risk estimate was nearly identical across the 3 studies (~1.3 per each additional copy of the *F11* minor allele). In contrast, we did not observe significant association between VTE occurrence and *SERPINC1 rs*2227589 or *GP6 rs*1613662 (Pro219Ser), though the risk estimate observed in CHS for the *GP6* variant was not dissimilar from that reported by Bezemer et al [9]. Our risk estimates for the A19911G variant of the prothrombin gene (*rs*3136516) [25, 26], the HR2 haplotype [27] and *rs*3753305 and *rs*4524/*rs*932619 variants of the factor V gene [8], and the *GC* promoter haplotype and *rs*2069915 variants of the protein C gene [8,28], were also comparable to previous reports. In contrast, we were unable to confirm VTE associations for haplotypes or SNPs of the protein C receptor [29,30], vitamin K epoxide reductase [31], factor VII, interleukin-10, or interleukin 1-related genes [10,32,33].

Other data suggest a lower thrombotic risk associated with Factor V Leiden among subjects 65 and older from CHS compared to middle-aged subjects from the ARIC study [35]. On the other hand, the risk of VTE associated with G20210A was higher in CHS (OR 2.98; 95% CI 1.00 – 8.85) than ARIC (OR 1.04; 95%CI 0.31 – 3.50) [36]. A similar pattern of association between CHS and ARIC has been reported for the alpha-fibrinogen Thr312Ala variant [37], which is in moderate linkage disequilibrium (r-squared 0.5) with the *rs*207006 variant associated with increased risk of VTE reported here and elsewhere [8].

Study strengths and limitations

The strengths of the current analysis include a comprehensive analysis of common patterns of genetic variation across our candidate genes and the use of a prospective study design that included all cases (both fatal and non-fatal) that occurred during follow-up. Several potential limitations of the current study should also be noted. Statistical power was limited to detect modest effect sizes, even for common variants. For example, a power analysis of the primary endpoint assuming minor allele frequency of 0.20 and an additive mode of inheritance indicates that we have >80% power to detect a relative risk of 1.4, but only ~30% power to detect a relative risk of 1.2. Because of the community-based study design, VTE cases that were diagnosed and managed solely as outpatients and rapidly fatal VTE events among non-hospitalized community residents were potentially missed. The relatively small numbers of African-American VTE cases or those with recurrent (as opposed to first-time) VTE events

limited our ability to assess heterogeneity of SNP effects among these subgroups. As with any indirect association study using common tagSNPs, we cannot distinguish which SNP is the true functional variant(s) and we also cannot exclude the possibility of associations with rare variants within our candidate genes. It is important to note that only persons 65 years and older are included in CHS. Certain SNPs with null findings for VTE in the current study might have been a result of survivor bias in CHS. This may explain why we were unable to reproduce some of the findings from previous studies that included younger VTE cases [9]. Likewise, additional assessment of any newly observed genetic associations in CHS require additional confirmation in independent study samples that include younger individuals in order to determine the generalizability of our findings to the community.

In summary, our findings suggest that several common coagulation gene variants may be related to risk of VTE in older adults. Further epidemiologic studies are needed to validate these findings in other populations and assess the functional molecular mechanisms. Ultimately, a better understanding of the risk factors for venous thrombosis may improve prevention and treatment of VTE in high risk individuals and groups of patients, such as older adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline characteristics of European-American and African-American CHS participants

Characteristic	European-American	African-American
Number	4,547	839
Mean age, years (range)	73 (65–98)	73 (65–93)
Female sex	2,580 (57)	523 (62)
Current smokers	497 (12)	131 (9)
Body mass index (kg/m^2)	26.4 ± 4.5	28.5 ± 5.6
Diagnosis of cancer	689 (15)	74 (9)
Estrogen use*	314 (12)	37 (7)
Warfarin use	70 (2)	18 (2)
Prevalent CVD†	1,085 (24)	227 (27)
VTE event during follow-up	144 (3)	40 (5)
First event	120	33
Recurrent event	24	7

Data are presented as number (%) or mean ± standard deviation, unless otherwise indicated.

*% of current estrogen use among women only.

†Self-reported myocardial infarction, stroke, angina, transient ischemic attack, claudication, or re-vascularization procedure.

Table 2

Candidate genes, number of tagSNPs tested per gene, and global gene-based p-value for association with VTE risk*

Gene Symbol	Description	# SNPs tested	# SNPs $p<0.05$	Individual SNPS with $p<0.05$	Global gene-based *P*-value
HABP2	Hyaluronan binding protein 2	29	3	rs3862019, rs1885436, rs6585234	<0.001
F2R	Coagulation factor II receptor	6	4	rs253061, rs153311, rs37248, rs2227744	0.01
PLAUR	Plasminogen activator, urokinase receptor	11	2	rs344782, rs344781	0.02
F13A1	Coagulation factor XIII, A subunit	29	7	rs3024409, rs3024422, rs3024405, rs5988, rs2274393, rs5982, rs2326707	0.04
F8	Coagulation factor VIII	3	1	rs1800291	0.06
TFPI	Tissue factor pathway inhibitor	4	1	rs3755248	0.13
SERPINA5	Protein C inhibitor	8	1	rs7070	0.13
F11	Coagulation factor XI	7	3	rs2289252, rs3756008, rs3733403	0.15
PLG	Plasminogen	5	2	rs13231, rs4252105	0.15
FGA	Fibrinogen alpha chain	6	3	rs2070006, rs6050, rs2070016	0.15
F5	Coagulation factor V	5	2	rs2239854, rs3753305	0.17
CPB2	Thrombin-activatible fibrinolysis inhibitor (carboxypeptidase B2)	8	1	rs17844008	0.30

* Table lists only those genes (of 51 genes tested) that had at least one tagSNP nominally associated with risk of VTE

Table 3

Candidate gene tagSNPs significantly associated with risk of venous thrombo-embolism at a false discovery rate of 0.20

SNP rs #	Candidate Gene	Chromosome	Position	Location	MAF	Hazard ratio (95% CI)	p-value	q-value
rs3024409	*F13A1*	6	6168293	intron	0.301	1.66 (1.31–2.11)	0.00001	0.003
rs6585234	*HABP2*	10	115320638	intron	0.180	0.49 (0.33–0.73)	0.00009	0.008
rs253061	*F2R*	5	76049756	intron	0.261	0.56 (0.41–0.77)	0.0001	0.008
rs3862019	*HABP2*	10	115318940	intron	0.352	0.62 (0.48–0.82)	0.0004	0.023
rs344782	*PLAUR*	19	48864730	intron	0.469	0.66 (0.52–0.84)	0.0007	0.033
rs153311	*F2R*	5	76063579	intron	0.249	0.64 (0.47–0.88)	0.003	0.128

MAF = minor allele frequency; UTR = untranslated region; CI = confidence interval

Table 4

Estimated mean coagulation factor activity, by *HABP2* rs3862019 genotype or *F8 rs*1800291 genotype*

	*HABP2 rs*3862019			*F8 rs*1800291		
Plasma Assay	**N**	**Estimated mean (95%CI)**	**P-value***	**N**	**Estimated mean (95%CI)**	**P-value***
Factor VII:C (u/dL)	G/G = 1891	123 (122–124)	--			
	G/T = 2327	123 (122–124)	0.82			
	T/T = 840	123 (121–125)	0.82			
Factor VIII:C (U/dL)	G/G = 1820	124 (122–126)	--	*Women*		--
	G/T = 2064	125 (123–126)	0.67	G/G = 1698	126 (124–127)	0.01
	T/T = 600	121 (118–124)	0.04	G/T = 688	121 (118–124)	0.08
				T/T = 91	118 (111–126)	
				Men		
				G = 1582	119 (117–120)	--
				T = 292	114 (110–118)	0.04
Factor IX:C (U/dL)	G/G = 140	121 (116–125)	--			
	G/T = 154	119 (115–123)	0.62			
	T/T = 50	111 (106–117)	0.02			
Factor X:C (U/dL)	G/G = 140	122 (118–125)	--			
	G/T = 154	119 (116–123)	0.31			
	T/T = 50	111 (106–117)	0.003			
Fibrinogen (mg/dL)	G/G = 1898	323 (320–326)	--			
	G/T = 2341	323 (321–327)	0.63			
	T/T = 843	322 (317–326)	0.66			
Plasminogen (mg/dL)	G/G = 140	106 (103–109)	--			
	G/T = 154	107 (104–110)	0.66			
	T/T = 50	100 (96–105)	0.02			

* adjusted for age, sex, race, and BMI

* P-values are shown separately for the comparison of each genotype group (heterozygotes or rare homozygotes) to the reference genotype group (common homozygotes).